DEVELOPMENT AND CHARECTERIZATION OF SOY LECITHIN AND PALM OIL BASED ORGANOGELS

A THESIS SUBMITTED IN PARTIAL FULFILLMENT

OF THE REQUIREMENTS FOR THE DEGREE OF

BACHELOR OF TECHNOLOGY

IN

BIO-MEDICAL ENGINEERING

SUBMITTED BY

NIROD BARAN

109BM0004

UNDER THE GUIDANCE OF

Dr. KUNAL PAL

DEPARTMENT OF BIOTECHNOLOGY AND MEDICAL ENGINEEIRNG

NATIONAL INSTITUTE OF TECHNOLOGY

ROURKELA-769008

DEPARTMENT OF BIOTECHNOLOGY & MEDICAL ENGINEERING,

NATIONAL INSTITUTE OF TECHNOLOGY-ROURKELA

Dated: 7th May, 2013

CERTIFICATE

This is to certify that the thesis entitled "**DEVELOPMENT AND CHARACTERIZATION OF SOY LECITHIN AND PALM OIL BASED ORGANOGELS**" submitted by **Mr. Nirod Baran** in partial fulfillment for the requirements for the award of Bachelor of Technology Degree in Biotechnology at National Institute of Technology, Rourkela is an authentic work carried out by him under the supervision of the undersigned.

To the best of my knowledge, the matter embodied in the thesis has not been submitted to any other University / Institute for the award of any Degree or Diploma.

(Dr. KUNAL PAL)

Assistant Professor

ACKNOWLEDGEMENTS

I would like to express my deep sense of gratitude and respect to our supervisor, Dr. Kunal Pal, Assistant Professor, Department of Biotechnology and medical Engineering, National Institute of Technology Rourkela for his excellent guidance, suggestions and constructive criticism. He has been very kind, supportive and patient to me while suggesting the outlines of the project and has also been very helpful in the successful completion of the same. I thank him for his overall support.

Last but not the least; I would like to extend my heartfelt gratitude to the Ph.D scholar, Mr. Vinay Kumar Singh, Mr. Sai Sateesh Sagiri and Ms. Beauty Behera, Department of Biotechnology and Medical Engineering, National Institute of Technology, Rourkela for their support and guidance. His helping nature and suggestion has helped me to complete this present work.

Date: Nirod Baran

Abbreviations

SL- Soy Lecithin

PO- Palm Oil

DW- Distilled Water

MZ- Metronidazole

CPDR- Cumulative Percent Drug Release

NCIM- National Collection of Industrial Microorganisms

RH- Relative Humidity

BFM- Bright Field Microscopy

RT- Room Temperature

MW- Molecular Weight

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

Preparation and characterization of soy lecithin (SL) and palm oil (PO) based organogels have been reported in this study. The optimization of the composition of the organogels was carried out by varying the proportions of SL, PO and water. Microscopic studies suggested the presence of aqueous phase either as fluid filled fibers or spherical droplets or both, depending on the composition of the organogels. FTIR study indicated strong intermolecular hydrogen bonding amongst the organogel components. The pHs of the organogels was found to be ~ 5.75 and were hemocompatible. The release of metronidazole (MZ; model drug) suggested diffusion mediated drug release. MZ loaded organogels have shown good antimicrobial property against *B. subtilis* and *E. coli*.

CHAPTER 1

INTRODUCTION

1. INTRODUCTION

Organogels are semisolid formulations which consist of apolar solvent as the liquid phase. They are often reported to be thermo-reversible and may be attributed to the physical interactions amongst the gel components [1-3]. Permanent organogels have also been reported [2]. Organogels have been found to be inherently thermodynamically stable below their gellation temperature. They possesses viscoelastic properties which is suitable for the development of topical/ transdermal formulations [1]. The properties of organogels may be tailored by incorporating water within its structure [4]. Sagiri et. al. (2012) showed that by varying the composition of organogels, it is possible to alter the drug release profile from the organogels. Due to the above-mentioned properties, there has been an increased research to device controlled drug delivery systems based on organogels [5].

Lecithin appears as a combination of zwitter ionic phospholipids (namely, phosphotidyl choline, phosphotidyl ethanolamine and phosphotidyl inositol), which forms either spherical or ellipsoidal reverse micellar structures when added to oil [5]. Addition of water into these apolar solutions results in the many-fold increase in the viscosity [6]. If the composition of the formulation is correct, the tri-phasic system forms organogels else remain as liquid mixtures [7]. Lecithin organogels have been reported to be thermo-reversible in nature and have a gel-to-sol transition of ~40°C [8]. The lecithin based organogels have also been reported to be viscoelastic in nature, an essential entity for the development of topical formulations [8].

Palm oil (PO) is extracted from the mesocarps and kernels of the fruits of the palm tree *Elaesis guineenis* and is rich in C16 and C18 fatty acids [9-10]. PO is rich in saturated fatty acids and has been found to be highly stable against oxidation. Due to this reason, PO has evolved as a suitable candidate to be used for topical formulations. Sakeena et. al. (2010) has reported the use of PO

ester based nanoemulsion of ketoprofen for topical application [11]. Owen et. al. (2000) has developed contraceptive gel using PO [12].

Taking inspiration from the above, it seems quite justified to develop PO and lecithin based organogels. Hence, an attempt was made to develop and characterize PO and soy lecithin (SL) based organogels. The developed organogels were tested as matrices for controlled delivery of metronidazole (MZ).

CHAPTER 2
LITERATURE REVIEW

2. Literature Review

A semi-solid formulation having an external solvent and apolar phase immobilized within a three dimensional networked structure is known as gel [3]. The organogels are bi-continuous systems consisting of gelators, apolar and polar solvent[1]. The gelator molecule undergoes physical or chemical transformations to form fibrous structures which get entangled with each other to form three-dimensional networked structure. The three-dimensional networked structure, immobilize the flow of external apolar phase [2].

Lecithin is a zwitter ionic phospholipid with two alkyl tails, when added to oil forms spherical or ellipsoidal reverse micelles. It is having three phospholipids i.e. phosphotidyl choline (PC), phosphotidyl ethanolamine (PE) and phosphotidyl inositol (PI) [7]. Different polar solvents such as glycerol, formamide and ethylene glycol, in addition to water have been studied to check the ability to induce organogel. The infra red results reveal that molecules of both the gel-forming solvents and non-gel-forming ones are attached to the lecithin phosphate group through hydrogen bonds [13].

Lecithin organogel is having a unique property of micelle formation. The micellar aggregates, much like the polymer molecules, overlap, interpenetrate, entangle, thus forming a temporal three-dimensional network that brings about viscoelastic properties. For this reason, the micellar system is in a jelly-like state [14]. It is widely used in everyday life, including human and animal food, medicine, cosmetics and manifold industrial applications due to the formation of the lipid matrix which plays a key role in the cellular metabolism[14].

Some organic solvents e.g. ether, linear, branched and cyclic alkanes, esters and amines have been used with lecithin in order to form organogels [14-15]. But due to toxicity of these apolar solvent recently oils such as palm oil, sun flower oil, soybean oil, mustard oil have been used to form organogels [3, 16].

Palm oil is made up mostly of glyceridic materials with some non glyceridic materials in small or trace quantities. Triglycerides and fatty acids composition triglycerides form the major component and bulk of the glyceridic material present in the palm oil with small amount of monoglycerides. Fatty acids chains present in the chain and in the structure [9-10]. So thus it makes palm oil a good apolar solvent in the preparation of organogel and also in the drug delivery.

The phase behavior of lecithin in n-decane employing water as the polar solvent has been discussed [14]. At first, with the addition of water, the thickening effect is observed at a certain specific molar ratio of water to lecithin. After this threshold concentration, further addition of water leads to a sharp increase in the viscosity and the formation of organogel [5].

The topical administration of drugs cutaneous and percutaneous drug delivery has recently got high importance as they are having easy noninvasive administration, local enhanced transdermal delivery, avoidance of local gastrointestinal toxicity and delivery benefits [17]. In a recent development, phospholipids in addition with some other additives such as water or apolar solvent have been shown to provide a very promising topical drug delivery medium known as lecithin organogels [17].

SL organogels have generated considerable interest over the years as a potential topical drug delivery vehicle [18]. One of the important applications of SL organogel is topical delivery. Due to existence of aqueous and organic phase, larger interfacial area, well defined structure, entrapment of solutes within the gel matrix and ability to form micells make it good topical drug delivery vehicle.

CHAPTER 3

MATERIALS AND METHODS

3. MATERIALS AND METHODS

3.1. MATERIALS

Soy lecithin (SL) was purchased from Otto India Pvt. Ltd., Kokata, India. Edible PO was purchased from the local market. Trisodium citrate was purchased from Loba Chemie Pvt. Ltd., Mumbai, India. Nutrient agar and dialysis tubing (MW cutoff: 60 kDa) were procured from Himedia, Mumbai, India. Microbial cultures of *Bacillus subtilis* (NCIM 2699) and *Escheratia coli* (NCIM 2563) were obtained from National Collection of Industrial Microorganisms (NCIM), Pune, India. MZ was received as a gift from Aarti drugs, Mumbai, India. Goat blood was collected from the local butcher shop. Double distilled water (DW) was used throughout the studies.

3.2. METHODS

3.2.1. Preparation of organogels

SL and PO based organogels were prepared by fluid fiber mechanism. The optimization of the organogels formation was carried out by varying the proportions of SL, DW and PO. A ternary phase diagram was plotted to have an overview of organogel forming area. The development of organogels was carried out as per the method reported by our group for the development of span 80/ tween 80 and SL based organogels with slight modifications [16, 19]. In short, accurately weighed SL was taken in culture tube and was dissolved in specified weight of PO (apolar solvent). The mixture was subsequently incubated at 70 ^{o}C for 5 min in a water-bath. Specified amount of DW, maintained at 70 ^{o}C, was added drop-wise to the SL-PO solution with constant vortexing to obtain a homogeneous mixture. The homogenous mixture was allowed to cool to room temperature (RT, 25 ^{o}C). The formation of gel was confirmed by tube inversion method.

3.2.2. Oraganoleptic Evaluation

Freshly prepared organogels were evaluated for organoleptic properties (e.g. odor, color, taste, appearance and texture) [20].

3.2.3. Stability studies

3.2.3.1. Accelerated stability Test

Freeze-thaw method was used to predict the long-term stability of the organogels. The gels were incubated alternatively in the water bath (maintained at 70 ^{o}C) and temperature controlled cabinet (maintained at - 4 ^{o}C) for 15 min each. The procedure was repeated for five cycles. After each cycle, gels were inspected for any signs of destabilization. The samples were regarded to be stable if they survived the harsh conditions for 5 cycles [19].

3.2.3.2. Intermediate stability test

The stability of the pharmaceutical products may be carried out by incubating the samples at a particular environment for a longer time period. International Conference on Harmonisation (ICH) guideline of stability of pharmaceutical products indicates the storage of the products at 30°C ± 2°C/65% RH ± 5% RH for 6 months (intermediate stability test) [21].

3.2.4. Microscopic studies

The microstructures of the gels were studied in detail by bright field microscopy (BFM; LEICA-DM 750 equipped with ICC 50-HD camera), phase contrast microscopy (PCM; Carl Zeiss, Model HBO 50, Germany) and scanning electron microscopy (SEM; Jeol JSM-6480LV, Japan) [22-23]. The BFM and PCM were carried out by making thin smears of the organogels. The thin

smears were dried in an oven at 45 ^{o}C for 12 h and were reanalyzed under PCM. The SEM studies were conducted by converting the organogels into xerogels as per the reported literature [24]. The xerogels were sputter-coated with platinum before the SEM studies [25].

3.2.5. pH measurement

The pH of the optimized organogels was measured using a digital ATC pH meter (EI instruments, model no- 132E) [26].

3.2.6. FTIR spectroscopy

The functional group identification and interaction, if any, amongst the components of the developed gels were studied by ATR-FTIR spectrophotometer (AlphaE ATR-FTIR, Bruker, USA). The scanning was done in the range of 4000 cm^{-1} to 500 cm^{-1} [27].

3.2.7. Disintegration studies

Tablet disintegration apparatus (Electronics, Model 901, Mumbai, India) was used to study the disintegration behavior of the organogels. The tests were conducted as per the reported literature [28]. In short, pellets of organogels (~1 g) were prepared by incubating the organogels at -20 ^{o}C for 30 min. The test was carried out in two disintegration fluids to simulate the gastric (SGF; 0.01 N HCl, pH=2.0) and intestinal (SIF; phosphate buffer saline of pH=6.8) conditions at 37 ±1 ºC. The total time taken for the complete disintegration of the organogels was noted as the disintegration time [28].

3.2.8. Gel Sol Transition Test

Gel-sol transition temperature was found out by incubating the organogels in a water bath, whose temperature was varied from 27-50 ºC. The temperature, at which the gels started to flow, when the glass vials were inverted, was noted as the gel-sol transition.

3.2.9. Hemocompatibility studies

The hemocompatibility study was carried out to find out the extent of hemolysis of the blood cells in the presence of the leachants of the developed organogels. The method has been described in details elsewhere [29]. The method deals with the incubation of the leachants of the organogels with the goat blood and analyzing the % hemolysis of the goat blood. The % hemolysis was calculated as per the formula given below [30-31]:

$$\%\ Hemolysis = \frac{OD_{test} - OD_{Negative}}{OD_{positive} - OD_{Negative}} \times 100$$

where, OD_{Test} = optical density of test sample, $OD_{Positive}$ = optical density of +ve control and $OD_{Negative}$= optical density of -ve control.

3.2.10. *In-vitro* Drug Release

In-vitro drug release was carried out using a 2-compartment modified Franz's diffusion cell as per the previously reported literature [32]. In short, accurately weighed ~1 gm of MZ containing organogels were taken in the donor chamber. 50 ml of DW was used as the dissolution medium and was kept in the receptor chamber. The receptor volume was stirred at 100 rpm at constant temperature 37 ± 1 °C. Previously activated dialysis membrane of MW cut-off - 60 kDa, obtained from Himedia, Mumbai, was used as the semipermeable membrane. The release study

was conducted for a period of 12 h. The whole receptor volume was changed periodically (15 min for first 1hr, 30 min for the next 2 h and 1 h for the next 9 h) with fresh DW. A portion of the replaced DW was further analyzed under UV- spectrophotometer (UV 3200 double beam, Labindia) at a wavelength of 321 nm [21]. The drug release studies were carried out in duplicate [33-34].

3.2.11. Antimicrobial assay

Antimicrobial assay was performed using bore well method. The antimicrobial efficacy of the MZ loaded organogels were studied against *Bacillus subtilis* (gram positive bacteria) and *Escherichia coli* (gram negative bacteria). Nutrient agar was used as a culture media for the study. 0.1 g of sample were accommodated in the wells of diameter 5 mm, which was bored using a sterilized borer.1 ml of nutrient broth cell suspension containing 10^6 to10^7 cfu/ml of bacteria was spread on the solid nutrient agar Petri plates using a sterilized spreader. The zone of inhibition was measured after the plated were incubated for 24 h at 37 ± 1 ℃.

CHAPTER 4

RESULTS

AND

DISCUSSIONS

4. RESULTS AND DISCUSSIONS

4.1. Preparation of Organogels

SL and PO based organogels were developed by varying the proportions of SL, PO and DW. The organogels were formed by fluid filled fiber mechanism [3]. Depending on the composition of the mixture of SL, PO and DW, the mixture either formed organogels or remained as liquid mixtures [35]. It was further confirmed by tube inversion method [35]. Depending on the composition of the organogels, the color of the developed organogels varied from cream color to dark brown color. The developed organogels were opaque in nature and were non-birefringent [3]. Apart from the organogels, emulsions were also formed when the concentration of SL was in between 10 % and 30 %. Figure 1 shows the compositions of the mixtures of the SL, PO and DW which resulted in the formation of organogels and emulsions. The organogels formation was observed when the concentrations of SL, DW and PO were in the range of ~43 % - ~80 %, ~17% - 55% and ~2% to 20%, respectively. Emulsions were formed when the concentrations of SL, DW and PO were in the range of ~10 % - ~25 %, ~45 % – ~60 % and ~25% to ~35% w/w (Figure 2).

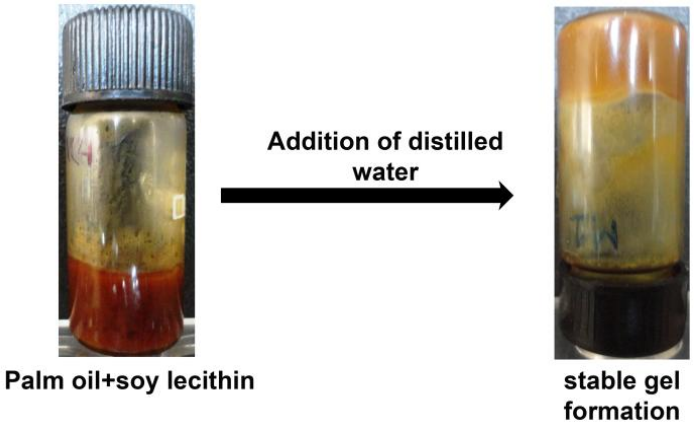

Figure 1: Mechanism of organogel and emulsion formation

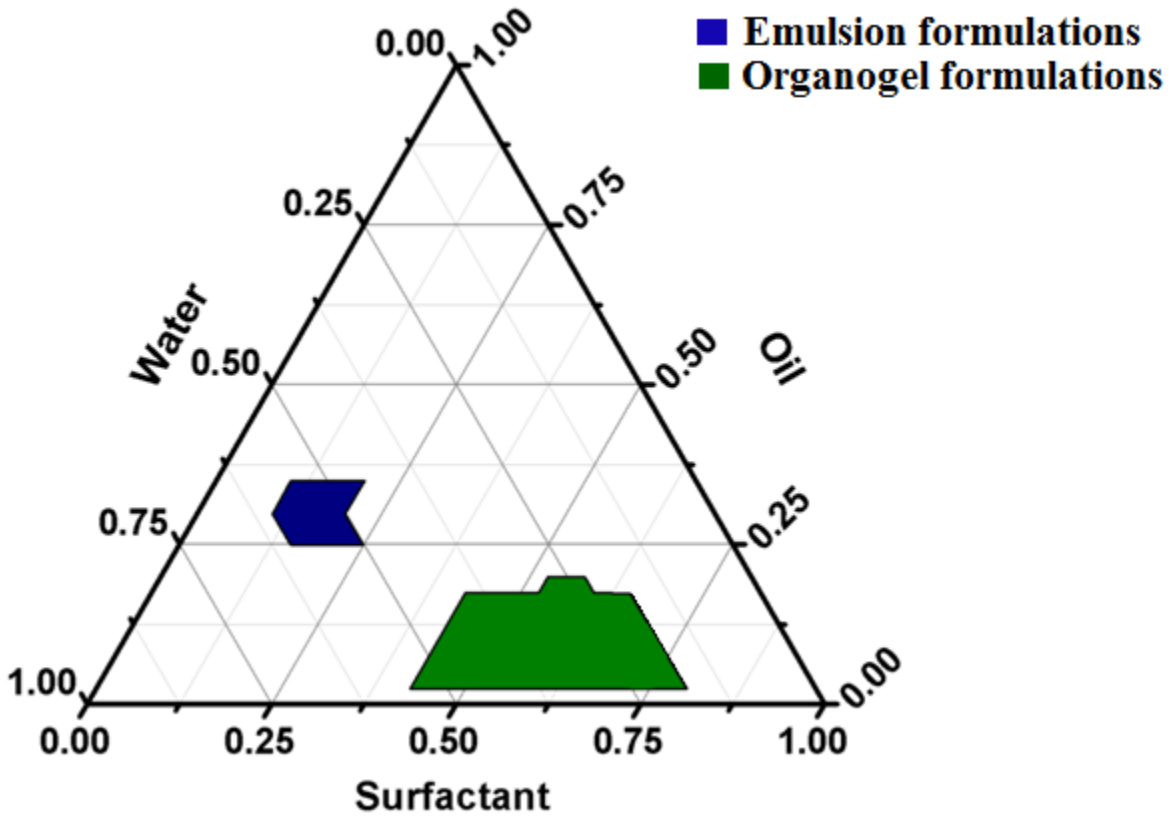

Figure 2: Mapping of the compositions forming organogels and emulsions

4.2. Organoleptic Evaluation

The color of the developed organogels was found to be varying from cream to brown in color. The emulsions were light brown in color. The consistencies of the organogels were improved with the increase in SL concentration. All the samples were found to be oily to touch and possessed pleasant odor.

4.3. Stability Studies

4.3.1. Accelerated Stability Test

Accelerated stability test was conducted by freeze-thaw method to have an understanding about the long-term stability of the organogels. The samples were observed for any signs of destabilization (e.g. phase separation, change in color or odor) during the course of the study. All the emulsions failed the accelerated stability test and hence were not studied further. Some of the developed organogels formulations have failed the test (marked in red, Figure 3) The formulations failed to maintain their structural integrity and started to flow. Rest of the organogel formulations have passed test. From the triplot it was clear that the organogels which failed the accelerated stability test were having compositions near to the critical gellation concentration (figure 3). 5 organogel formulations were selected from the stable formulations as representative samples for further characterization. 1 % MZ was incorporated within these selected organogels. The compositions of the representative organogels and their corresponding drug incorporated organogels have been provided in table 1.

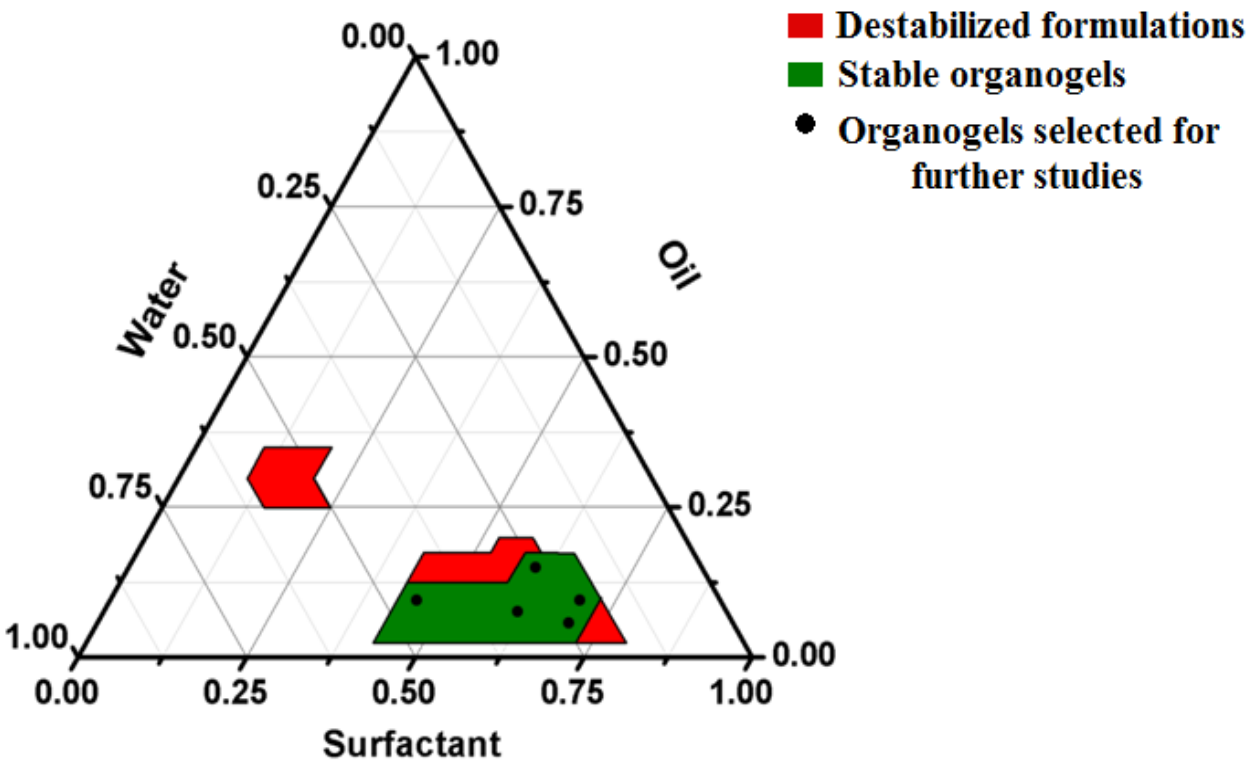

Figure 3: Mapping of the compositions of the formulations after accelerated stability test

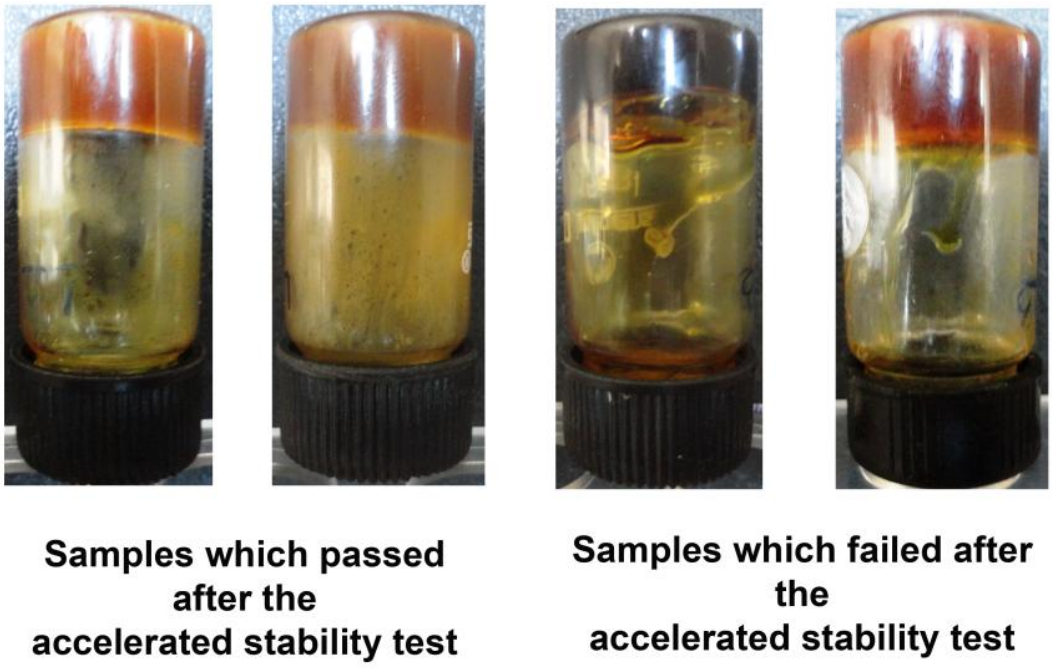

Figure 4: Representative organogel formulations after the accelerated stability test. (a) Stable organogel and (b) and Destabilized organogel

Table 1: Composition of the organogels used for further analysis

Formulations	SL (% w/w)	PO (% w/w)	DW (% w/w)	MZ (% w/w)
PLG1	**45**	**10**	**45**	**-**
PLG1M	**45**	**10**	**44**	**1**
PLG2	**60**	**7.5**	**32.5**	**-**
PLG2M	**60**	**7.5**	**31.5**	**1**
PLG3	**60**	**15**	**25**	**-**
PLG3M	**60**	**15**	**24**	**1**
PLG4	**70**	**5**	**25**	**-**
PLG4M	**70**	**5**	**24**	**1**
PLG5	**70**	**10**	**20**	**-**
PLG5M	**70**	**10**	**19**	**1**

4.3.2. Long term stability test

All the selected formulations were kept for long-term stability test. The organogels were incubated at 30 ℃ ± 2 ℃ and 75% ±5% RH for 12 months. All the samples passed the test with no change in the physical appearance i.e. change in color, phase separation or syneresis of the organogels.

4.4. Microscopic studies

The microstructures of the organogels have been shown in figure 5. When the concentration of the SL was lowest (~45 %), uniformly distributed spherical-shaped droplets were observed. The

distribution pattern of the dispersed droplets became uneven when the concentration of SL was increased. With the further increase in the SL to ~70 %, a network of fiber-like structures was observed. This may be attributed to the supramolecular structure of the lecithin molecules [14].

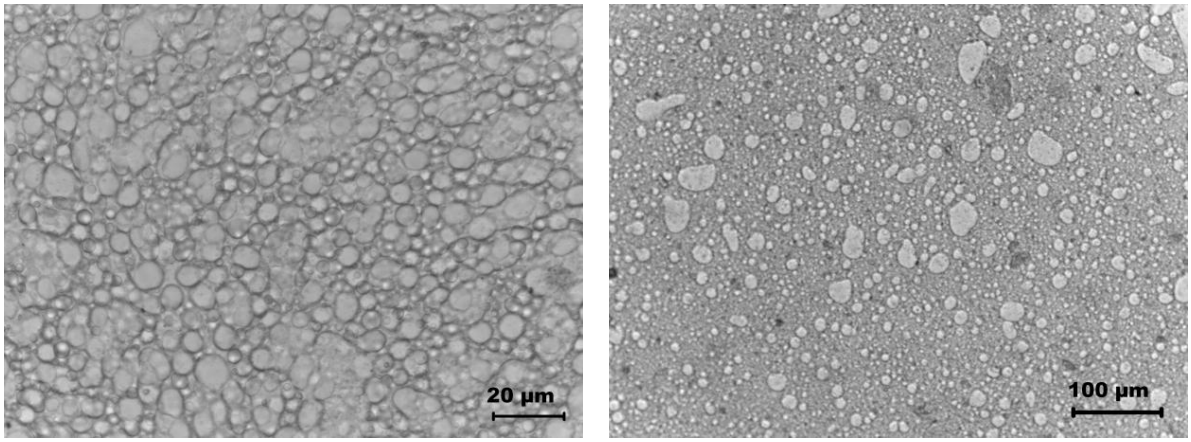

Figure 5: Bright field micrographs of the organogels showing the dispersed phase structures.

4.5. pH measurement

The normal pH of the human skin varies in the range of 5.5-7.0. The pH of the developed organogels formulations were found to be in range of 5.6 to 5.75 (table 3). The results indicated that the formulations might be used for topical applications.

Table 2: pH value of the selected organogels

S. No.	Sample	pH value
1	PLG1	5.61 ± 0.12
2	PLG2	5.63 ± 0.16
3	PLG3	5.71 ± 0.18
4	PLG4	5.75 ± 0.11
5	PLG5	5.73 ± 0.10

4.6. FTIR spectroscopy

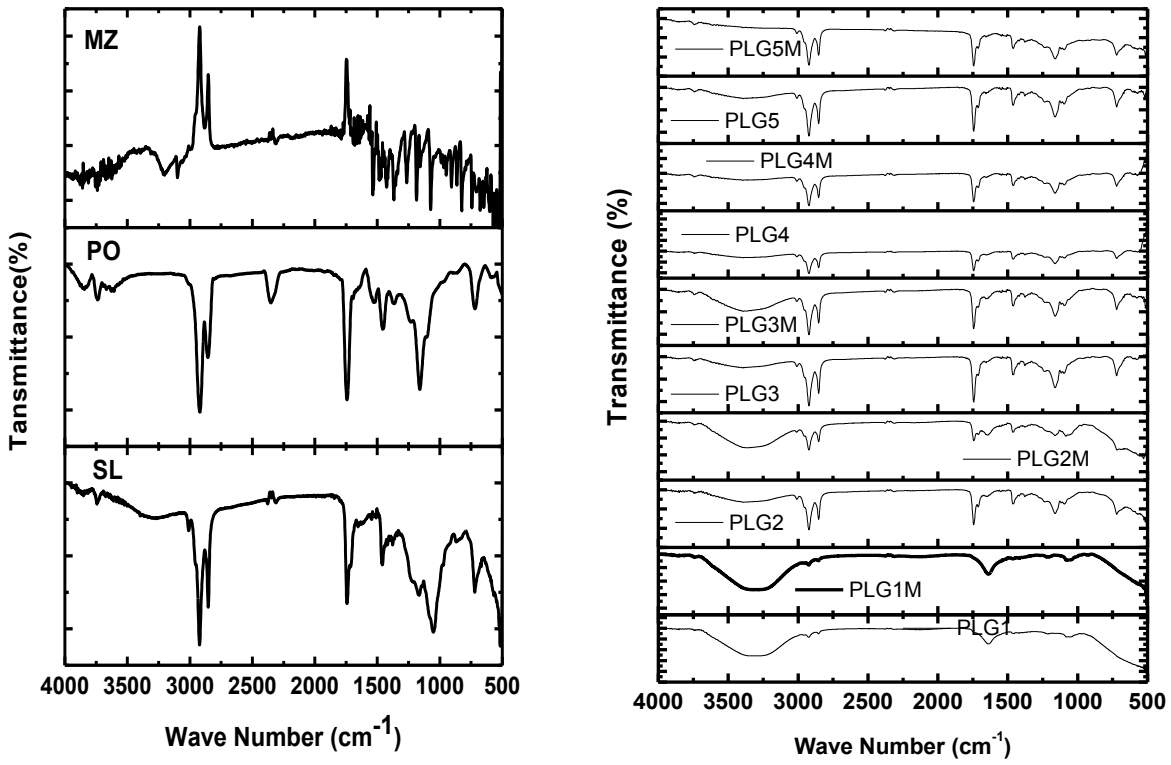

Figure 6: FTIR study of raw material and their MZ loaded gels

FTIR spectrograms of the raw materials (SL and PO) and the organogels (blank and drug loaded) have been shown in figure 9. SL showed a broad peak at ~3600 cm^{-1} due to the -OH stretching vibrations [16]. The absorption peaks at ~ 2848 cm^{-1} and ~ 1461 cm^{-1} may be associated to the C-H stretching and bending vibrations, respectively, of the alkanes. The absorption peak at ~ 1745 cm^{-1} may be associated to the -CO stretch of the carbonyl groups. The peaks at ~ 721 cm^{-1} may be associated to the CH rocking due to the alkanes [14, 16] . PO has shown the absorption peaks at ~2920 cm^{-1} and ~2850 cm^{-1} which was associated to the CH stretching in CH_3 and CH_2 of alkanes [36]. The absorption peaks at ~ 1470 cm^{-1} was due to the CH_2 scissoring vibrations [37]. The peak at ~1743 cm^{-1} may be associated with the stretching vibration of CO group of triglycerides present in PO [38]. MZ has shown absorption peak at ~3220 cm^{-1} which may be associated with the OH stretching. The absorption peak at 3,100 cm^{-1} may be attributed to C–H stretching vibration. The absorption peak in the range of 1400–1535 cm^{-1} may be accounted to the functional groups present in the imidazole ring [39]. The peak at 1348 cm^{-1} was attributed to the NO symmetrical stretch of imidazole. The absorption peak at 1732 cm^{-1} may be associated with the C=C double bond [40]. The FTIR spectra of the organogels have shown the presence of absorption peaks corresponding to the raw materials. A slight shift was observed in few absorption peaks which may be explained due to the change in the chemical environment around the components of the organogels. A broad peak was observed at ~ 3350 cm^{-1} which suggested the presence of strong intra-molecular/ intermolecular hydrogen bonding amongst the organogel components. The increase in the intensity as compared to the raw materials indicated an increase in the hydrogen bonding amongst the gel components [7, 16]. No extra peak was observed in the MZ loaded organogels. This may due to the fact that MZ was present in very minute

concentrations and the peaks associated with MZ were subsided because of the intense peaks of the other components.

4.7. Disintegration Test

Table 9 shows the time required for the complete disintegration of the organogels in SGF and SIF. In general, the disintegration time of the organogels in both the simulated fluids was dependent on the concentration of the DW. There was an increase in the disintegration time of the organogels with the decrease in the DW concentration except PLG4. The disintegration time of PLG4 was highest amongst all the gels and did not follow the trend. The results indicated that PLG4 may be used as a carrier for controlled delivery of bioactive agents for prolonged period of time whereas PLG1 may be a good carrier for the delivery of drugs where quicker release of drugs are required [24, 41].

Table 3: Disintegration test of the selected gels

	Disintegration time	
Formulations	**pH 1.2**	**pH 6.8**
PLG1	58 min	1 h 11 min
PLG2	2 h 2 min	3 h 25 min
PLG3	1 h 58 min	3 h 25 min
PLG4	3 h 20 min	4 h
PLG5	2 h 30 min	2 h 21 min

4.8. Gel Sol Transition:

Table 7: Gel-sol transition of the optimized organogels

Formulations	25^0C	30^0C	35^0C	40^0C	45^0C	50^0C	55^0C
PLG 1	√	√	√	√	X	X	X
PLG 2	√	√	√	√	√	√	X
PLG 3	√	√	√	√	√	X	X
PLG 4	√	√	√	√	√	√	X
PLG 5	√	√	√	√	√	X	X

Gel-sol transition showed the melting point of the developed organogels was in the range of 45 to 55^0C (table 7). The MP of the gels was found to be increased with SL concentration suggesting presence of surfactant might have increased the structural integrity. At the same time it may also be concluded that presence of water also imparted an increase in the structural organization of the gel samples. PLG 2 has shown higher melting point as compared to PLG 3 which may be associated to the higher DW concentration in PLG2 as compared to PLG3. Both PLG2 and PLG3 possessed equal SL concentration. So it might be the DW which might have increased the inter molecular hydrogen bonding.

4.9. Hemocompatibility Test

The results of the hemocompatibility test have been tabulated in the table 12. The results suggested that the organogels were highly hemocompatible in nature.

Table 5: Hemocompatibilty test

Sample Name	% Hemolysis
PLG1	3.05 ± 0.08
PLG2	1.42 ± 0.04
PLG3	3.55 ± 0.09
PLG4	0.00 ± 0.00
PLG5	0.00 ± 0.00

4.10. *In- vitro* drug release

The drug release profiles of MZ from the organogels have been shown in figure 7. The results suggested that the release of the drug was dependent on the composition of the organogels. The cumulative percentage drug release (CPDR) of MZ from the organogels was in the order of PLG1M > PLG2M > PLG3M > PLG4M > PLG5M. This indicated that the CPDR of the drug was lowered due to the combined effect of the decrease in the concentration of DW and an increase in the concentration of SL.

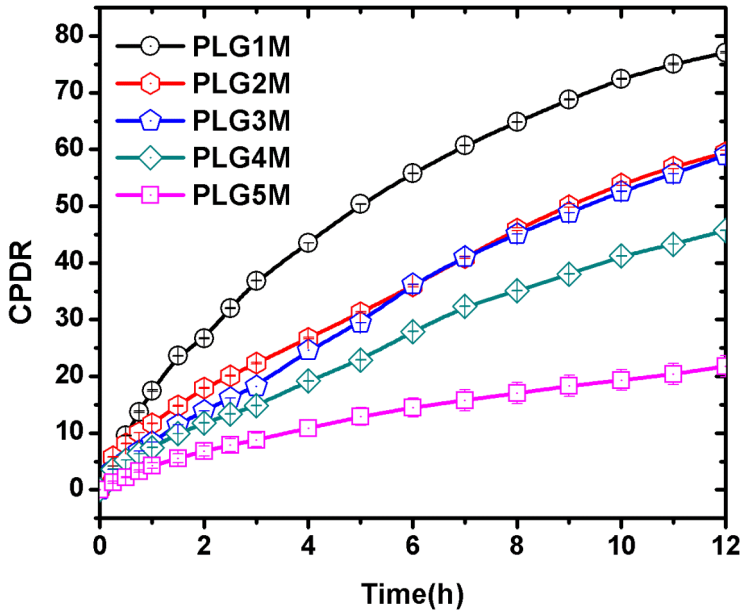

Figure 7: CPDR of the developed gels

4.11. Antimicrobial assay

The efficiency of the drug incorporated organogels was studied against *B. subtilis* (a model gram positive bacterium) and *E. coli* (a model gram negative bacterium). The zone of inhibition was measured after 24 h of incubation at 37 ± 1 °C. Metrogyl, the marketed MZ gel, was taken as the positive control whereas the blank gels were treated as the negative controls. The results indicated that the developed organogels have shown good antimicrobial efficacy against the gram positive and gram negative bacterium and the antimicrobial efficiency was comparable to the marketed formulations (Figure 8-9).

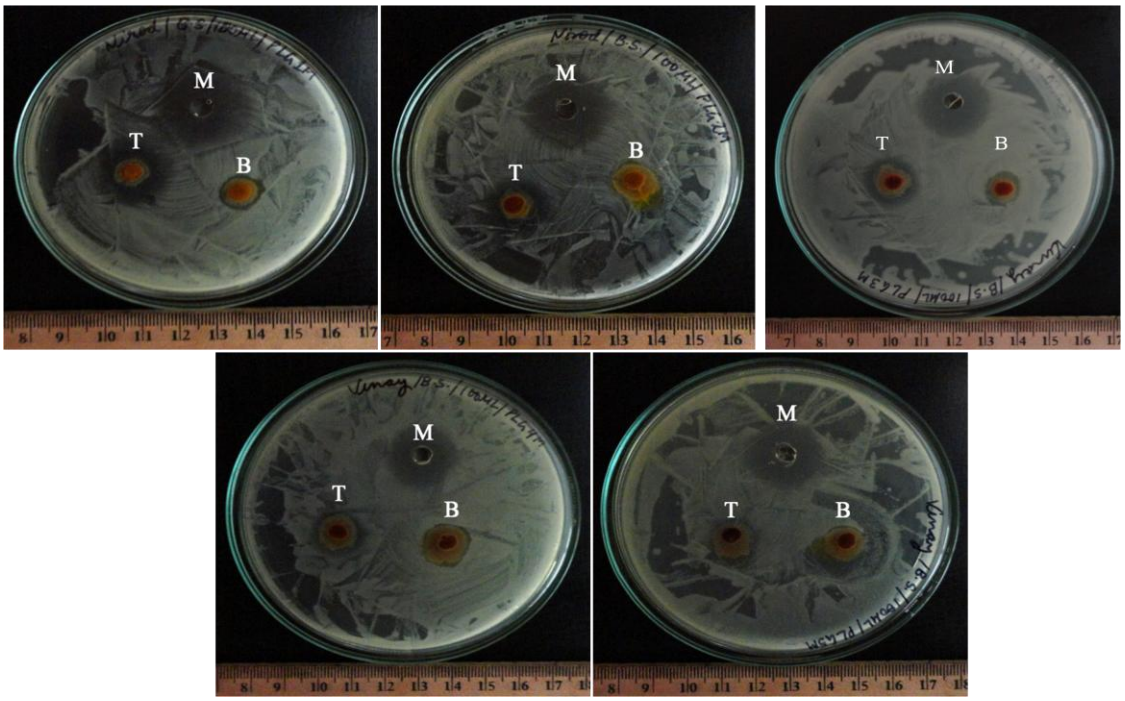

Figure 8: Antimicrobial efficacy of the organogels against *E. coli*

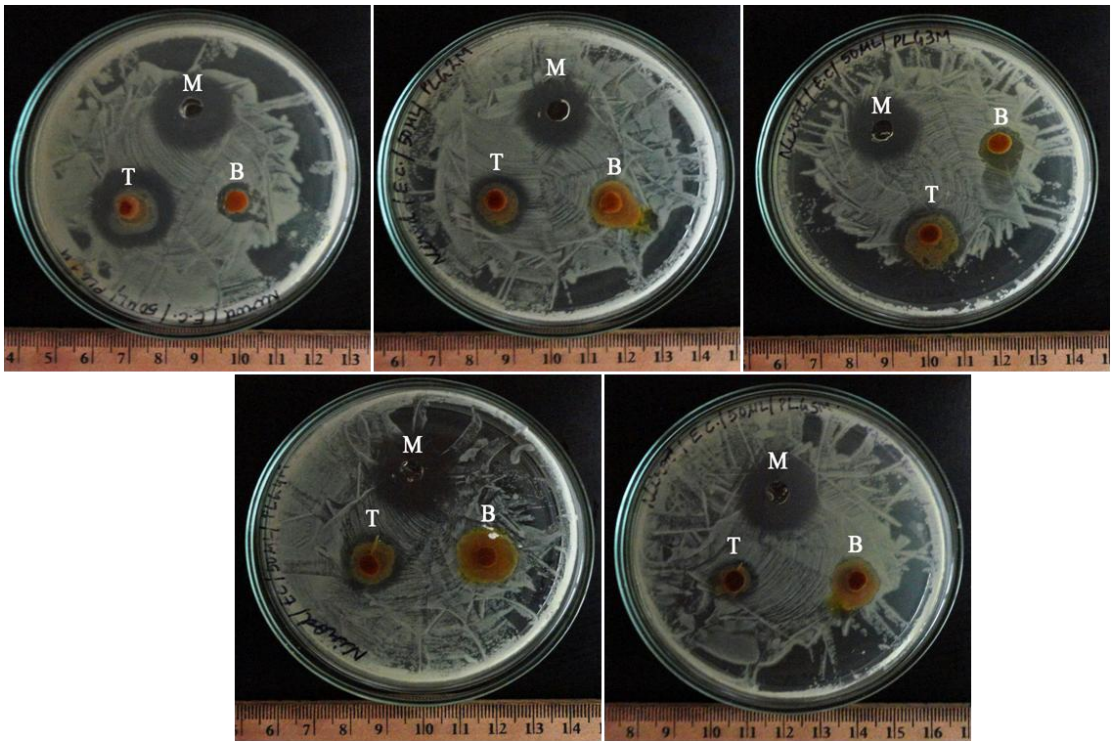

Figure 9: Antimicrobial efficacy of the organogels against *B. subtilis*

CHAPTER 5
CONCLUSION

5. CONCLUSION

This study reports the successful development of the SL and PO based organogels. The preliminary study suggested that the developed systems may be used as potential carriers for bioactive agents. The organogels were easy to prepare and had shown excellent thermal stability. The disintegration test suggested the developed systems may be used either as a controlled release system or as a quick release system, depending on the composition of the organogel. The developed organogels were found to have good spreadability and viscosity profile desired for the development of topical formulations. The organogels were found to be highly biocompatible. The antimicrobial assay of the MZ-loaded organogels has shown comparable antimicrobial as against Metrogyl®. In short, it may be concluded that the developed organogels may be used as carriers for antimicrobial bioactive agents for topical application.

CHAPTER 6
BIBLIOGRAPHY

6. BIBLIOGRAPHY

1. Gupta, S., et al., *Organogel: a viable alternative for existing carrier system.* system, 2011. **4**: p. 5.
2. Vintiloiu, A. and J.-C. Leroux, *Organogels and their use in drug delivery—a review.* Journal of Controlled Release, 2008. **125**(3): p. 179-192.
3. Sahoo, S., et al., *Organogels: Properties and Applications in drug delivery.* Designed Monomers and Polymers, 2011. **14**(2): p. 95-108.
4. Behera, B., et al., *Span-60-based organogels as probable matrices for transdermal/topical delivery systems.* Journal of Applied Polymer Science, 2012. **125**(2): p. 852-863.
5. Kumar, R. and O.P. Katare, *Lecithin organogels as a potential phospholipid-structured system for topical drug delivery: a review.* AAPS PharmSciTech, 2005. **6**(2): p. 298-310.
6. Scartazzini, R. and P.L. Luisi, *Organogels from lecithins.* The Journal of Physical Chemistry, 1988. **92**(3): p. 829-833.
7. Pearson, R.H. and I. Pascher, *The molecular structure of lecithin dihydrate.* 1979.
8. Szuhaj, B.F., *Lecithins: sources, manufacture & uses*. Vol. 12. 1989: Amer Oil Chemists Society.
9. Sreekala, M., M. Kumaran, and S. Thomas, *Oil palm fibers: Morphology, chemical composition, surface modification, and mechanical properties.* Journal of Applied Polymer Science, 1998. **66**(5): p. 821-835.
10. Sambanthamurthi, R., K. Sundram, and T. YewAi, *Chemistry and biochemistry of palm oil.* Progress in Lipid Research, 2000. **39**(6): p. 507-558.
11. Sakeena, M., et al., *Formulation and in vitro evaluation of ketoprofen in palm oil esters nanoemulsion for topical delivery.* Journal of Oleo Science, 2010. **59**(4): p. 223-228.
12. Owen, D.H., J.J. Peters, and D.F. Katz, *Rheological properties of contraceptive gels.* Contraception, 2000. **62**(6): p. 321-326.
13. Shchipunov, Y.A. and E.V. Shumilina, *Lecithin bridging by hydrogen bonds in the organogel.* Materials Science and Engineering: C, 1995. **3**(1): p. 43-50.

14. Shchipunov, Y.A., *Lecithin organogel: a micellar system with unique properties.* Colloids and Surfaces A: Physicochemical and Engineering Aspects, 2001. **183**: p. 541-554.
15. Luisi, P., et al., *Organogels from water-in-oil microemulsions.* Colloid & Polymer Science, 1990. **268**(4): p. 356-374.
16. Satapathy, D., et al., *Sunflower-oil-based lecithin organogels as matrices for controlled drug delivery.* Journal of Applied Polymer Science, 2012.
17. Patil, K.D., S. Bakliwal, and S. Pawar, *Organogel: topical and transdermal drug delivery system.* Int J Pharm Res Dev, 2011. **3**(6): p. 58-66.
18. Lawrence, M.J. and G.D. Rees, *Microemulsion-based media as novel drug delivery systems.* Advanced drug delivery reviews, 2000. **45**(1): p. 89-121.
19. Sagiri, S.S., et al., *Effect of Composition on the Properties of Tween-80–Span-80-Based Organogels.* Designed Monomers and Polymers, 2012. **15**(3): p. 253-273.
20. Agrawal, V., et al., *Preparation and Evaluation of Tubular Micelles of Pluronic Lecithin Organogel for Transdermal Delivery of Sumatriptan.* AAPS PharmSciTech, 2010. **11**(4): p. 1718-1725.
21. Singh, V.K., et al., *Castor oil and sorbitan monopalmitate based organogel as a probable matrix for controlled drug delivery.* Journal of Applied Polymer Science, 2013.
22. Song, J. and R.I. Hollingsworth, *Synthesis, conformational analysis, and phase characterization of a versatile self-assembling monoglucosyl diacylglycerol analog.* Journal of the American Chemical Society, 1999. **121**(9): p. 1851-1861.
23. Novales, B., et al., *Self-assembly of fatty acids and hydroxyl derivative salts.* Langmuir, 2008. **24**(1): p. 62-68.
24. Shah, D.K., et al., *Development of olive oil based organogels using sorbitan monopalmitate and sorbitan monostearate: A comparative study.* Journal of Applied Polymer Science, 2012.
25. Rogers, M.A., et al., *A novel cryo-SEM technique for imaging vegetable oil based organogels.* Journal of the American Oil Chemists' Society, 2007. **84**(10): p. 899-906.
26. Haering, G. and P.L. Luisi, *Hydrocarbon gels from water-in-oil microemulsions.* The Journal of Physical Chemistry, 1986. **90**(22): p. 5892-5895.

27. Belgamwar, V., et al., *Pluronic lecithin organogel.* Asian Journal of Pharmaceutics, 2008. **2**(3): p. 134.
28. Murata, Y., et al., *Properties of pectin gel bead modified with pectin hydrolysate and chitosan.* Advances in Polymer Technology, 2005. **24**(1): p. 46-50.
29. Behera, B., et al., *Span-60-based organogels as probable matrices for transdermal/topical delivery systems.* Journal of Applied Polymer Science, 2012.
30. Bhattacharya, C., et al., *Development of span 80–tween 80 based fluid-filled organogels as a matrix for drug delivery.* Journal of pharmacy & bioallied sciences, 2012. **4**(2): p. 155.
31. Pal, K., A. Banthia, and D. Majumdar, *Biomedical evaluation of polyvinyl alcohol–gelatin esterified hydrogel for wound dressing.* Journal of Materials Science: Materials in Medicine, 2007. **18**(9): p. 1889-1894.
32. Willimann, H., et al., *Lecithin organogel as matrix for transdermal transport of drugs.* Journal of pharmaceutical sciences, 1992. **81**(9): p. 871-874.
33. Tokuyama, H. and Y. Kato, *Preparation of thermosensitive polymeric organogels and their drug release behaviors.* European Polymer Journal, 2010. **46**(2): p. 277-282.
34. Couffin-Hoarau, A.C., et al., *In situ-forming pharmaceutical organogels based on the self-assembly of L-alanine derivatives.* Pharmaceutical research, 2004. **21**(3): p. 454-457.
35. Suzuki, M., et al., *Supramolecular organogel formation triggered by acid–base interaction in two-component system consisting of L-lysine derivative and aliphatic acids.* New J. Chem., 2007. **31**(9): p. 1654-1660.
36. Ifuku, S., et al., *Preparation and characterization of redox cellulose Langmuir–Blodgett films containing a ferrocene derivative.* Journal of Polymer Science Part A: Polymer Chemistry, 2005. **43**(21): p. 5023-5031.
37. Atek, D. and N. Belhaneche-Bensemra, *FTIR investigation of the specific migration of additives from rigid poly (vinyl chloride).* European polymer journal, 2005. **41**(4): p. 707-714.
38. Pérez-Mateos, M., P. Montero, and M.C. Gómez-Guillén, *Formulation and stability of biodegradable films made from cod gelatin and sunflower oil blends.* Food Hydrocolloids, 2009. **23**(1): p. 53-61.

39. Yuan, J. and M. Liu, *Chiral molecular assemblies from a novel achiral amphiphilic 2-(heptadecyl) naphtha [2, 3] imidazole through interfacial coordination.* Journal of the American Chemical Society, 2003. **125**(17): p. 5051-5056.
40. Cristescu, R., et al., *Functional polyethylene glycol derivatives nanostructured thin films synthesized by matrix-assisted pulsed laser evaporation.* Applied Surface Science, 2009. **255**(24): p. 9873-9876.
41. Iwanaga, K., et al., *Characterization of organogel as a novel oral controlled release formulation for lipophilic compounds.* International journal of pharmaceutics, 2010. **388**(1): p. 123-128.